

Preliminary Amendment

Page 5 of 6

Applicant(s): Eric T. Baldwin et al.

Serial No. 09/896,580

Filed: June 29, 2001

For: CRYSTALLIZATION AND STRUCTURE OF *STAPHYLOCOCCUS AUREUS* PEPTIDE DEFORMYLASE

Remarks

The specification has been amended to correct obvious typographical errors in citations. An incomplete citation on page 49, lines 21-22 (Van Duyne, Standaert, 1993) has also been corrected. The correct citation is fully supported at, for example, page 41 of U.S. Provisional Application Serial No. 60/215,550, filed June 30, 2000, which was incorporated by reference on page 1, lines 8-10 of the present application.

The specification has been amended at page 12, line 10, to clarify that mutations are indicated in Figure 3 by boxed amino acids, as is fully supported at, for example, page 48, lines 24-29. The specification has also been amended at page 12, line 13 to indicate that the cylinders represent helices and the arrows represent sheets in Figure 5, which is fully supported at, for example, page 23, lines 14-22.

Substitute drawing sheets are being submitted herewith in accordance with MPEP §§608.02(h) and 608.02(v) to correct obvious errors. Specifically, in Figure 1 the subscripts have been clarified as would be obvious to one of skill in the art, and the clarification is supported, for example, in Rajagopalan et al., *Biochemistry*, 36:13910-18 (1997), which was cited on page 24, lines 17-18 of the present application and incorporated by reference at page 64, lines 32-35 of the present application. In Figure 5, the wording "Red-helix, Green-sheet" has been deleted. In Figure 10, the spelling of "*S. aureus*" has been corrected.

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Page 6 of 6

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Conclusion

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

Respectfully submitted for

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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Serial No.: 09/896,580

Docket No.: 6317.N

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Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 12, line 1, has been amended as follows:

Figure 3 lists the amino acid sequences of peptide deformylases from various species of bacteria including *Staphylococcus aureus* peptide deformylase (pdf) with C-terminal 6xHis tag (SEQ ID NO: 1); *Escherichia coli* pdf (SEQ ID NO:2); *Haemophilis influenzae* pdf (SEQ ID NO:3); *Bacillus subtilis* (SEQ ID NO:4); and *Mycoplasma pneumoniae* (SEQ ID NO:5); and *Staphylococcus aureus* def1 gene (a related but inactive form of the protein, also called Pseudo pdf) (SEQ ID NO:6). Alignments were generated from GCG SeqLab (Wisconsin Package Version 10.1, Genetics Computer Group, Madison, WI). The underlined residues show regions of importance to the activity of peptide deformylases. The [highlighted] boxed amino acids show mutations for *S. aureus* pdf (SEQ ID NO:1).

The paragraph beginning at page 12, line 13, has been amended as follows:

Figure 5 is a schematic secondary structure diagram of *S. aureus* pdf. The cylinders represent helices and the arrows represent sheets.

The paragraph beginning at page 37, line 18, has been amended as follows:

Thus, this method involves generating a preliminary model of a molecule or molecular complex whose structure coordinates are unknown, by orienting and positioning the relevant portion of *S. aureus* pdf or the *S. aureus* pdf/ligand complex according to Table 1 within the unit cell of the crystal of the unknown molecule or molecular complex so as best to account for the observed x-ray diffraction pattern of the crystal of the molecule or molecular complex whose

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structure is unknown. Phases can then be calculated from this model and combined with the observed x-ray diffraction pattern amplitudes to generate an electron density map of the structure whose coordinates are unknown. This, in turn, can be subjected to any well-known model building and structure refinement techniques to provide a final, accurate structure of the unknown crystallized molecule or molecular complex (E. Lattman, "Use of the Rotation and Translation Functions," in *Meth. Enzymol.*, 115:55-77 (1985); M.G. [Rossmann] Rossmann, ed., "The Molecular Replacement Method," *Int. Sci. Rev. Ser.*, No. 13, Gordon & Breach, New York (1972)).

The paragraph beginning at page 49, line 16, has been amended as follows:

For expression of selenomethionyl-Pdf, M9 glucose was utilized in 100 ml volumes containing ampicillin, thiamin, and PAS trace metal solution at 100 mg, 5 mg and 0.3 ml per liter of deionized water, respectively. Multiple shake flasks were used to attain the desired fermentation volume. Since JM109 is not a methionine auxotroph, incorporation of selenomethionine was accomplished through down regulation of methionine biosynthesis just prior to induction (Van Duyne, Standaert, *J. Mol. Biol.*, 229:105-124 (1993)). The culture was grown in 500 ml wide mouth fermentation flasks at 37°C with an agitation rate of 200 rpm until A600 reached ca. 0.5 unit. At this point, the following filter sterilized amino acids were added to achieve down-regulation. DL-selenomethione, L-lysine, L-threonine and L-phenylalanine were added to final concentrations of 120 micrograms/ml. L-leucine, L-isoleucine and L-valine were added to final concentrations of 60 micrograms/ml. After 15-20 minutes, protein expression was induced by the addition of filter sterilized IPTG added to a final concentration of 0.4 mM. Growth of the culture was continued as described for an additional 3 hours when A600 reached ca. 2 units. Cells were then harvested by centrifugation and stored at -80°C.

The paragraph beginning at page 63, line 3, has been amended as follows:

This data was used for the further refinement of the native pdf structure. The partially refined model derived from the MAD map was rotated to an arbitrary initial position, stripped of

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water and cations, and used for molecular replacement (XPLOR). The rotation solutions were filtered with PC-refinement (Brunger, *Acta Crystallogr.*, A46:46-[47]57 (1990)). The highest rotation function peak also resulted in the [hghest] highest PC-filtered peak (PC=0.194). The position of the rotated monomer was obtained by a translation search (again the highest peak in the map and 15.6 sigma above the mean). The solution obtained was consistent with the position of the molecule in the MAD map and had an initial R-factor of 39.6% for data from 20-2.5 Å resolution (9235 reflections). This structure was further refined with XPLOR positional refinement and waters and a Zinc atom incorporated into the model. The R-factor dropped to 21% with a Free-R-factor of just over 25%. A final cycle of refinement and rebuilding was employed using PROLSQ (Hendrickson et al., "Stereochemically restrained crystallographic least-squares refinement of macromolecule structures" in *Biomolecular Structure, Function and Evolution*, (R.Srinivasan, ed. 43-57) Pergamon Press, Oxford UK ([1980] 1981)) which resulted in a final R-factor of 18.62% for 16266 reflections, 10-2.0 Å resolution data. The final agreement statistics (Table 10) and Ramachandran plot revealed a well-refined structure and are included below. Additional statistics were generated with PROCHECK (Laskowski et al., *J. Appl. Cryst.*, 26:283-91 (1993)). A comparison of the initial MAD map and the final refined map was produced in CHAIN.

FIGURE 1

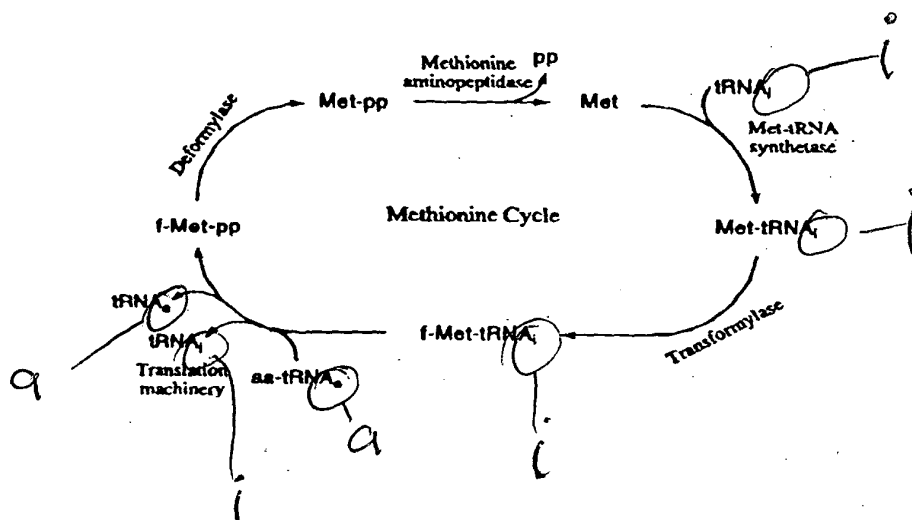


FIGURE 5

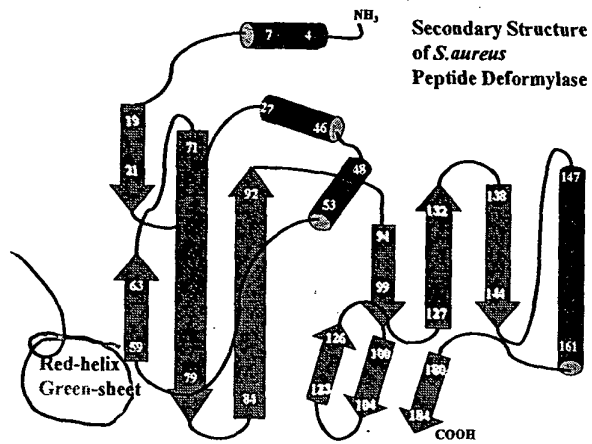


FIGURE 10

S.aureus MLTMKDIIRDGHLRQAAEIELPLT EEKETLIMREFLVNSQDEEIAKRYG
E.coli SV LKVAKPVEEV EIQRIVDMFETMY
S.aureus GVGLA~~P~~QINISKRMIAVLIPDDGSGKSYLVNPKIVS SVQ~~E~~AYLPTEGCL
E.coli GIGLA~~P~~QVDIHQRIIVIDVSEN LINPELLES GETGI EGCL
S.aureus VDDNVALVHRHNRI IKAKDIEGNDIQRLKGYPAIVFQHEIDHINGVMFYDHI
E.coli IPEQR LVPRAEKV IRALDRDGKPFLEAD~~E~~LIAICIQHMDHIVGKIFMDYL
S.aureus DKDHPLQPHTDAVEVHHH
E.coli SPLKQQRIRQKVEKLDRLK